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TECHNICAL MANUSCRIPT 464

A METHOD FOR PREPARING SMALL QUANTITIES OF C^{14}-LABELED STAPHYLOCOCCAL ENTEROTOXIN B

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DEPARTMENT OF THE ARMY
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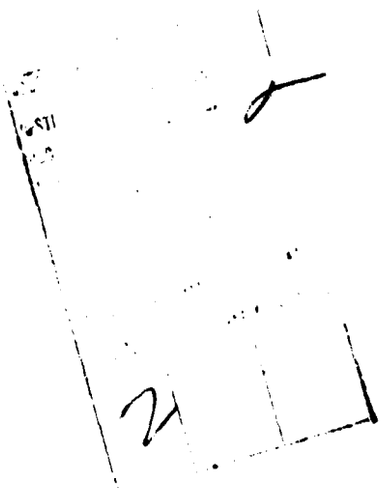

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Physical Science Division
BIOLOGICAL SCIENCES LABORATORY

Project 1B522301A059

June 1968

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

A procedure is described for production from Staphylococcus aureus of small amounts of radioisotopically labeled staphylococcal enterotoxin B for animal metabolism studies. The procedure is a modified scaled-down adaptation of the Schantz method of enterotoxin production. The organism is cultured on medium containing C^{14}-labeled yeast protein hydrolyzate, and the toxin is purified by adsorption on and elution from Amberlite CG-50 resin columns. The procedure is capable of yielding 12 to 16 mg of C^{14}-labeled enterotoxin B of high purity (96 to 100%) with an isotope concentration of 0.2 microcurie per mg.

I. INTRODUCTION

The preparation of highly purified staphylococcal enterotoxin B produced by Staphylococcus aureus has been described by Bergdoll, Sugiyama, and Dack,[1,2] Frea, McCoy, and Strong,[3] and Schantz et al.[4] A modified scaled-down adaptation of the Schantz procedure was developed to produce small amounts of radioisotopically labeled toxin for animal metabolism studies. The method consists of culturing the organism on medium containing C^{14}-labeled yeast protein hydrolyzate and subsequently purifying the toxin by adsorption on and elution from Amberlite CG-50 resin columns. The procedure is capable of yielding 12 to 16 mg of C^{14}-labeled enterotoxin B of high purity (96 to 100%) with an isotope concentration of 0.2 microcurie per mg.

II. MATERIALS AND METHODS

The small-scale procedure differs from the method of Schantz et al.[4] in three respects: (i) The growth medium for S. aureus was altered in an attempt to obtain higher toxin production by the culture in an apparatus designed to trap any volatile radioactive material produced by the bacteria, since toxin production in the presence of the trapping device was generally lower than in cultures grown in flasks stoppered with a cotton plug. (ii) The toxin was eluted from the first CG-50 resin column more selectively by using an eluent with a lower salt concentration. This was done to permit the volumes of the eluate fractions to be proportionately larger, a practical consideration necessitated by the small scale of the operation. The method of adsorption and elution of the toxin from the second resin column was essentially the same as that of the Schantz procedure. (iii) Fractionation of the enterotoxin on the carboxymethyl cellulose column was omitted because essentially pure toxin was obtained in the previous step.

The toxin was produced by culturing S. aureus 64-I-T (a high-toxin-producing strain derived from S-6) in medium containing 4 g of N-Z-Amine, Type A* and 0.4 g of yeast extract** in 100 ml of tap water. To this medium, 1.13 mg (800 μc/mg) of uniformly C^{14}-labeled yeast protein hydrolyzate*** were also added. The culture was incubated for 24 hours

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* Sheffield Chemical Co., Norwich, N.Y.
** Difco Laboratories, 920 Henry St., Detroit 1, Mich.
*** International Chemical and Nuclear Corp., 13332 E. Amar Road, City of Industry, Calif.

at 37 C with constant shaking on a wrist action shaker. Cultures normally produced 200 to 320 μg of toxin per ml. Escape of radioactive carbon dioxide into the atmosphere was prevented by continuously drawing the air above the culture through a cylinder of 0.5 N potassium hydroxide solution by means of a Gast air pump. At the end of the incubation period, the culture was adjusted to pH 7 and filtered through a Seitz filter. The filtrate was then dialyzed against 6 liters of water for 5 hours at room temperature, diluted with two volumes of water, and adjusted to pH 6.4. To the diluted filtrate, 1.2 g of Amberlite CG-50 resin* that had previously been exchanged with sodium ion and equilibrated with 0.05 M sodium phosphate at pH 6.4 were added. The toxin was quantitatively adsorbed on the resin after stirring the resin and filtrate mixture for 30 minutes at room temperature. The resin containing the adsorbed toxin was filtered into a 1 by 10 cm glass column and washed with an equal column volume of water. The column was eluted with 0.15 M sodium phosphate pH 6.8 at a flow rate of 0.3 ml per minute, and each of the collected fractions had a volume of 3 ml. The toxin was quantitatively eluted from the column when the effluent volume reached 50 to 100 ml. Toxin-containing fractions that could be identified by their strong ultraviolet absorption at 277 mμ were combined and dialyzed against 200 ml of 0.01 M phosphate buffer at pH 7.0 to reduce the salt concentration of the fractions. The volume was concentrated to approximately 10 ml by lyophilization, and the concentrated solution was again dialyzed against phosphate buffer at pH 6.8. The toxin solution was then added to a second column (1 by 10 cm) containing 1.2 g of CG-50 resin, which had been previously equilibrated with 0.05 M sodium phosphate solution at pH 6.8 and washed with water to remove the buffer salts. After adsorption of the toxin, the column was washed with an equal volume of water, and the toxin moved through the column with 0.15 M sodium phosphate solution at pH 6.8. The toxin in the final purified state emerged from the column in the volume of effluent between 5 and 20 ml. Yields equivalent to 40 to 60% overall recovery were obtained by this procedure. The amount of radioactivity in the product was determined by counting samples of toxin solution previously dried on small pieces of filter paper and placed in counting solution in a liquid scintillation spectrophotometer.** Appropriate corrections were made for adsorption and quenching. The product had a radioactivity of 0.2 μc/mg, which represented incorporation of 0.2 to 0.3% of the isotope from the medium.

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* Mallinckrodt Chemical Works, Second and Mallinckrodt St., St. Louis 7, Mo.

** Packard Tri-Carb Model 3003.

III. CRITERIA OF PURITY

The quantity of toxin present in the final product was determined by assay with the Oudin serological technique,[5,6] as modified by Silverman[7] and Schantz et al.[4] The value obtained by this technique divided by the nitrogen content of the toxin determined by micro-Kjeldahl analysis was 6.30, which compares closely with 6.22 stated by Schantz et al.[4] to be the theoretical toxin-to-nitrogen ratio for pure enterotoxin.

The purified toxin was exposed to antiserum from rabbits immunized with a toxin preparation of 20% purity according to the technique of Ouchterlony.[8] A single antigen-antibody line was observed in the presence of enterotoxin concentrations up to 1 mg/ml, which was identical to the precipitin line formed with monovalent antiserum (Fig. 1).

Figure 1. Precipitation Zone Formation by Rabbit Antiserum with Radioisotopic Enterotoxin B. The upper row of wells contains polyvalent antiserum prepared with 20% pure enterotoxin, the middle row plus the wells at the extreme top and bottom of the figure contain the enterotoxin, and the lower row contains monovalent toxin-specific antiserum.

A 0.28% solution of purified labeled enterotoxin in 0.5 M sodium phosphate solution, pH 6.6, was subjected to ultracentrifugation at 60,000 rpm at 20 C using a Spinco analytical ultracentrifuge Model E. The schlieren pattern exhibited by the solution was typical of a homogeneous substance. The sedimentation constant was determined to be 2.73 svedbergs, which agrees closely with the value of 2.84S calculated for the same concentration of purified enterotoxin prepared by the method of Schantz et al.[4]

As a final test, the labeled purified enterotoxin was injected into the saphenous vein of nine rhesus monkeys, 3 kg in weight, to determine if it had the same biological activity as the unlabeled toxin. Observations for emetic responses were made over a period of 5 hours. At a dose level of 1 μg of toxin per kg of body weight, two of three monkeys showed emesis; at a dose level of 0.33 μg per kg, one of three showed a response; and at a 0.1-μg dose, none of the three injected animals exhibited a reaction. These results indicate that the toxicity of the radioisotopic enterotoxin was not significantly different from that of the unlabeled substance.[4]

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13. ABSTRACT

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14. Key Words
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Staphylococcus aureus
Radioisotopes

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